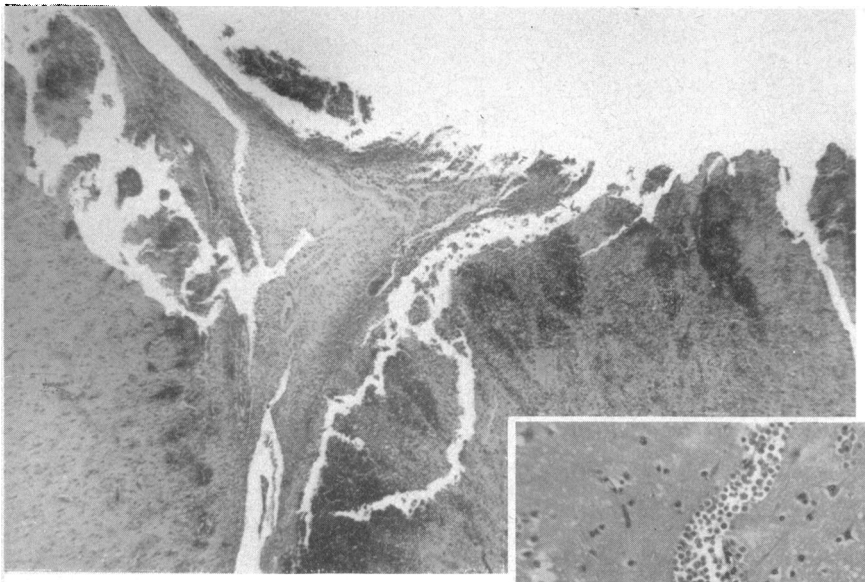

FIG. 1.—Photomicrograph of a coronal section through the olfactory sulcus of the frontal cortex just caudal to the olfactory bulb, showing the extensive meningeal exudate and disruption of the underlying cortex by haemorrhage and cellular infiltrate. (Haematoxylin and eosin. ×28.)

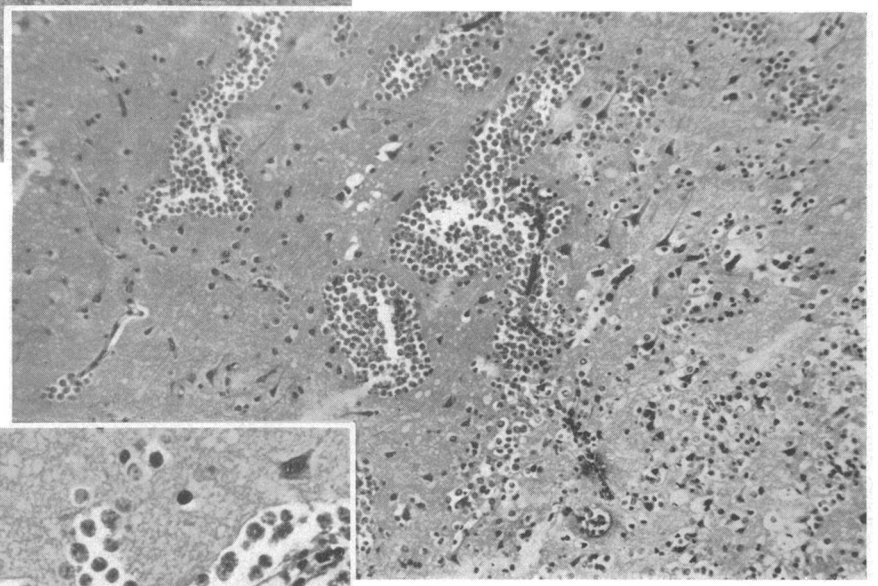

FIG. 2.—Photomicrograph of the same section as Fig. 1. The superficial cortical layer shown on the right of the photograph is almost completely disrupted by amoebae and inflammatory cells. In the deeper cortex to the left, amoebae are massed in perivascular spaces, and there is little inflammatory reaction. (Haematoxylin and eosin. ×115.)

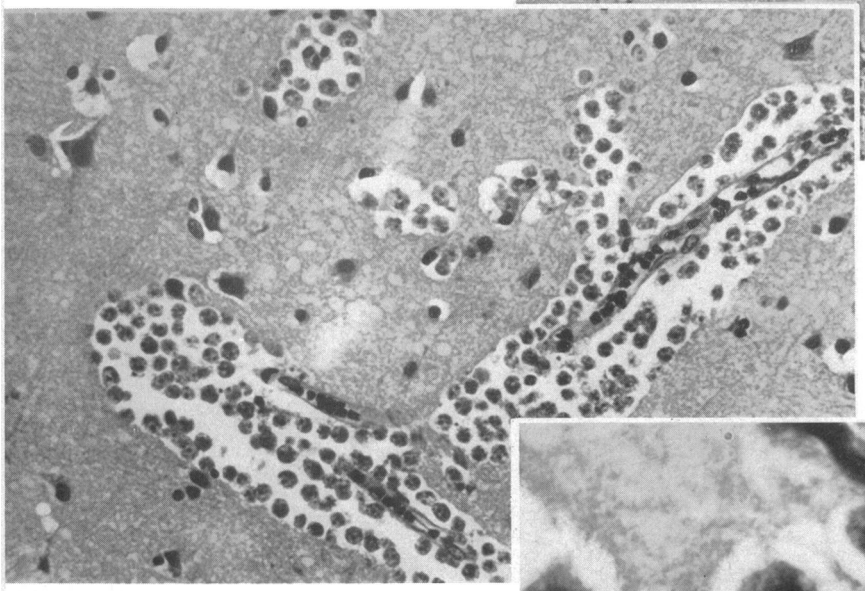

FIG. 3.—Photomicrograph of the deeper cortical layer from the same section as Fig. 1. Amoebae are massed in a branching perivascular space and many can be seen free in the brain substance or surrounding necrotic neurones. There is no inflammatory reaction. (Haematoxylin and eosin. ×285.)

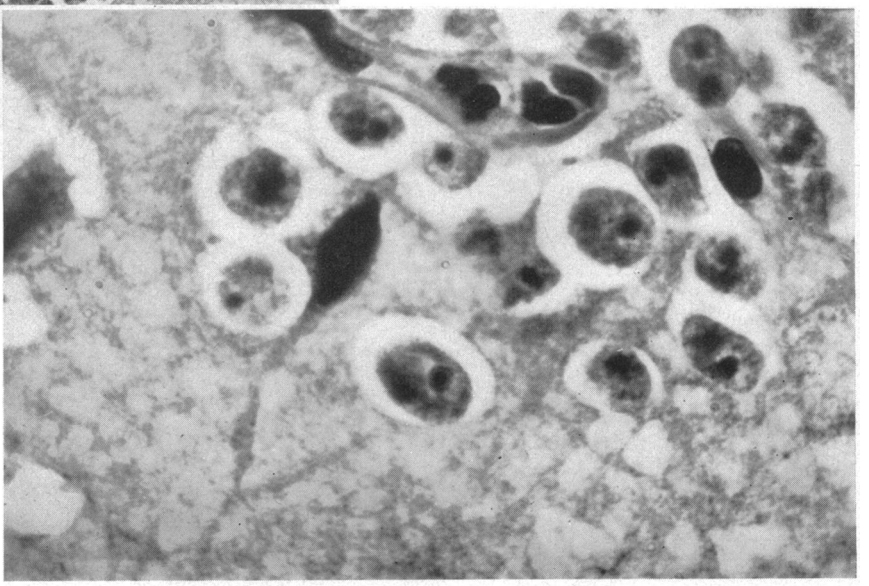

FIG. 4.—Photomicrograph of amoebae in a very small perivascular space, and free in the brain substance. The characteristic morphology of the organisms can be seen, the darker areas in the cytoplasm of several being ingested red blood cells. (Haematoxylin and eosin. ×1,135.)

urethra. Case 1 had been investigated thoroughly on three different occasions but not diagnosed, and had the diverticulum not remained filled at the time of the recent examination the nature of the complaint would have again been missed. In Case 2 the problem was of treatment and not of diagnosis. A more conservative approach was employed in the first instance in view of the proximity of the forthcoming confinement. In retrospect it is felt that the use of Ellik's procedure would have been less difficult than surgical excision in the presence of the considerable chronic inflammatory reaction. Case 3 in particular would tend to favour the congenital origin of the lesion.

As three cases of urethral diverticulum were diagnosed by me in a period of two years the condition is obviously more common than I suspected. This knowledge should lead to a more frequent diagnosis of the lesion in the future.

Summary

Diverticula of the female urethra have often been reported in America in the past 20 years, while reference to them in the British literature is still scanty.

Three cases of diverticulum are presented and discussed.

A short summary of the aetiology, symptomatology, and treatment is presented.

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Preliminary Communications

Acute Pyogenic Meningitis Probably Due to *Acanthamoeba* sp.: a Preliminary Report

[WITH SPECIAL PLATE]

Brit. med. J., 1965, 2, 740–742

In 1961 a 9-year-old boy from Port Augusta, a country town in South Australia, died at the Adelaide Children's Hospital with a meningitis pathologically unremarkable except for the presence in the brain and meninges of large numbers of amoebae, morphologically distinct from *Entamoeba histolytica*. No pathogenic organisms were cultured from the cerebrospinal fluid or meningeal exudate, but the clinical record and laboratory investigations were considered to be characteristic in all other respects of acute bacterial meningitis.

In 1965 three further patients from the same district died in similar circumstances, and this report is a brief account of the clinical and pathological manifestations, with a hypothesis on the aetiology and pathogenesis of what we believe to be a previously undescribed disease.

CASES 1, 2, AND 3

The clinical features of the first three patients, a 9-year-old boy and two girls both aged 8 years (Adelaide Children's Hospital Case Nos.: AU 992, AM 3871, and BD 2015), are so similar that they will be presented collectively. Before the onset of their fatal illness all were considered by their parents to be in good health, and no history of previous illnesses likely to be of aetiological significance was elicited by specific questioning. The first symptoms were noted on the fourth day before death, the children being described as lethargic and disinterested in their usual activities. On the following day all were feverish, obviously unwell, and complaining of severe headache, sore throat, and a blocked nose. Their local medical officers diagnosed an upper respiratory tract infection and started treatment with intramuscular and oral preparations of various antibiotics.

By the end of the third day, the patients' general condition having markedly deteriorated, with the onset of severe vomiting and an impaired level of consciousness, they were admitted to their local hospital with a provisional diagnosis of acute meningitis. This was confirmed by the finding of purulent cerebrospinal fluid on lumbar puncture, and was treated with glucose-saline fluids, chloramphenicol, penicillin, and sulphadiazine by the intravenous route. Deepening coma and signs of cardio-respiratory failure led to their transfer to the Adelaide Children's Hospital, where they all arrived moribund. Despite vigorous intravenous antibiotic and fluid therapy and attempts to relieve raised intracranial pressure they died shortly after admission.

Their cerebrospinal fluids contained predominantly neutrophil leucocytes varying from 400 to 8,000/c.mm., no micro-organisms were detected in stained smears, and cultures were unproductive of bacteria. However, all other features were so characteristic of fulminating bacterial meningitis that this was considered to be the nature of the disease, no other aetiological agent being seriously contemplated.

CASE 4

The clinical features of the fourth patient in this series, a 28-year-old man (Royal Adelaide Hospital Case No. 061501), are identical with those of the first three cases, except for the history of a sore throat and headache for two weeks before the onset of his major symptoms. He was transferred, in an equally moribund condition, from his local district hospital to the Royal Adelaide Hospital, where he died shortly after admission, death being considered due to acute meningitis of unknown aetiology.

PATHOLOGY

Post-mortem examinations were performed at four, two, five, and 48 hours after death (Adelaide Children's Hospital P.M. Nos: 8/61, 8/65, and 18/65; University of Adelaide Path. No. : 18208).

In all cases body development was normal, and no evidence of chronic disease was found in any organ. The gastro-intestinal tract, liver, and other abdominal viscera were not significantly abnormal. The lungs exhibited moderate oedema and vascular engorgement, with the addition in one instance of early bronchopneumonia. In three of the hearts the right-sided chambers were flabby and dilated, and the myocardium contained small foci of necrosis and inflammatory cell infiltration.

The skull and middle ears were normal. The brain was swollen only moderately, having flattened surface convolutions and partially obliterated sulci; there was no evidence of pressure coning. Meningeal veins were collapsed, superficial capillaries over the vertex engorged, and a few petechial haemorrhages were present in the pia mater and superficial cortical substance. A thin, creamy exudate was obvious in the basal subarachnoid cisterns, but elsewhere was not very apparent, except where it outlined a few of the sulcal grooves. In contrast to this appearance of rather minimal inflammation, the olfactory bulbs were very reddened, soft, and adherent by a mass of sticky exudate to the adjacent frontal cortex.

Microscopically the meningeal exudate consisted of about equal proportions of neutrophil leucocytes and chronic inflammatory cells, amongst which small, often degenerate amoebae were sparsely distributed. In the sulcal depths, and particularly in the Virchow-Robin spaces, the amoebae were better preserved and more clearly

discernible. They might be found clustered around blood-vessels in even the finest ramifications of these spaces, where they often appeared to have excited little or no inflammatory response. The cerebral substance in most areas appeared undisturbed except for a few necrotic neurones and the presence of occasional amoebae lying free within it. In striking contrast, the olfactory bulbs were almost completely disorganized by fibrino-purulent exudate and by haemorrhage from necrotic blood-vessels, and the adjacent frontal cortex was invaded to considerable depth by amoebae massed in hundreds in perivascular pathways or scattered diffusely through the brain substance (Special Plates, Figs. 1, 2, 3). Neutrophil leucocytes and red blood cells were scattered about in the necrotic and disintegrating cerebral tissue, and in many instances had been ingested by amoebae. The nasal mucous membrane subjacent to the cribriform plate (removed in only one case) was intensely inflamed, together with the ramifying filaments of the olfactory nerves, in which many amoebae could be identified.

In sections stained with haematoxylin and eosin the amoebae were distinguishable as irregularly circular bodies 6–9 μ in diameter, usually outlined by a clear surrounding zone 2 μ wide, but without any sign of an outer pellicle or differentiated ectoplasm. The coarsely granular and vacuolated cytoplasm stained a magenta colour, and might contain various ingested fragments. Its inner zone often appeared as several large almost confluent vacuoles surrounding the nuclear membrane. The usually single and centrally situated nucleus, 2 μ in diameter, consisted of a very fine, purple-staining nuclear membrane separated by a clear zone from an obvious, solid central nucleolus of the same colour and about half the diameter (Special Plate, Fig. 4). Material staining positively with the periodic-acid-Schiff technique (P.A.S.) was not present in any of the organisms, nor had any other special staining method so far proved superior to haematoxylin and eosin in demonstrating their morphology.

Cultures of the brain and meningeal exudate from all the cases yielded no bacteria, nor were tubercle bacilli, torula, or viruses isolated, where these were sought. In one instance the cellular sediment from cerebrospinal fluid removed from the patient before death was stained belatedly by us with iron-haematoxylin, and numerous shrunken, and probably degenerated, amoebae could be differentiated by their distinctive nucleus from surrounding inflammatory cells and macrophages.

Discussion

The features we have described leave us in no doubt that death in these four patients was primarily due to acute invasion of the meninges and brain by an unusual form of amoeba. Such a disease has not been reported in the literature, and in seeking to establish the identity and mode of entry of the organism in our material we have considered all the known amoebae, paying particular attention to those which are commensal or occasionally pathogenic in man and animals.

The only amoeba generally recognized as invading human tissue is *Entamoeba histolytica*. Meningitis as a complication of systemic infection by this amoeba is occasionally reported, but evidence of primary bowel invasion has always been present, and furthermore the morphology of the organism is quite distinct from that which we have described in our cases ; the total cell diameter is at least twice as great and the nucleus is dissimilar, consisting of a minute nucleolus surrounded by a very distinct nuclear membrane, on the inner surface of which the bulk of the nuclear chromatin is distributed as large granules. Moreover, glycogen vacuoles, usually present in the cytoplasm, stain positively with the P.A.S. technique, whereas in our material this was never observed.

Only two instances of human cerebral invasion by amoebae other than *Entamoeba histolytica* are recorded: Derrick (1948) described a case of overwhelming amoebiasis, including meningitis, which he attributed to *Iodamoeba bütschlii*. Kernohan *et al.* (1960) attributed a brain granuloma to the same organism, but offered no satisfactory explanation of its pathogenesis. In Derrick's case the invasion of human tissue by organisms normally non-pathogenic and commensal in the bowel was explained by the very debilitated state of the patient, and the meningitis was undoubtedly secondary to systemic spread of the organism following primary bowel invasion. Though his description of the amoebae is very similar to ours, we find it difficult to suggest such a pathogenesis in the present cases in the absence of bowel pathology, and even more difficult to postulate invasion of other sites in healthy individuals by such an organism.

This dissatisfaction having led us to consider other types of amoebae, our attention was drawn to recent experimental demonstration of the mammalian pathogenicity of the genus *Acanthamoeba* (or *Hartmanella*). This comprises many species of small, free-living amoebae, ubiquitously distributed in water and moist soil. Though their ecological relationships in soil biology had been widely studied, their pathogenicity to higher animals was unsuspected until it was demonstrated by Culbertson *et al.* (1959) and Culbertson (1961), who instilled cultures of *Acanthamoeba* sp. trophozoites into the noses of mice. Many of these animals died four or five days later with meningitis attributable in all respects to the introduced organism, which, after primarily invading the nasal mucous membrane, had extended via the olfactory nerves to the brain and meninges. Detailed comparison of the morphology of the amoebae in histological preparations of their animal and our human brains (personal communication) reveals a marked similarity. Several other features of our cases are comparable to animal infection by *Acanthamoeba* sp.: all the patients succumbed with an acute meningitis four to five days after the onset of severe symptoms of upper respiratory tract infection. Pathological investigation failed to elucidate a causative organism other than the amoebae, whose absence elsewhere in the body, overwhelming predominance in the olfactory bulbs and adjacent forebrain, and presence in the inflamed nasal submucosa and olfactory nerves of one patient, strongly suggest their invasion of the brain and meninges via the nasal mucosa.

While there must always be great reluctance to postulate causation of disease by organisms traditionally regarded as non-pathogenic, such occasions are not without precedent, and we consider that the evidence we have presented points to some species of *Acanthamoeba* as a causative agent of acute meningitis in humans.

If this hypothesis is tenable, then the geographically restricted occurrence of the cases suggests that a survey of sources of *Acanthamoeba* in this area and study of their animal pathogenicity might elucidate further their possible relationship to the human disease. These considerations are being investigated by one of us (R. F. C.), and the results, together with a more detailed account of the cases briefly reported here, will be the subject of a later publication.

Summary

Four cases of rapidly fatal pyogenic meningitis are described, all originating from the northern region of the Gulf of St. Vincent in South Australia.

Necropsy revealed a purulent meningeal exudate negative to conventional cultural methods but showing amoebae in sections of the brain and meninges.

The organism is described and differentiated from *Entamoeba histolytica*. Following a discussion of experimental infection with free-living amoebae, it is postulated that the organism is a member of the genus *Acanthamoeba*.

Strong evidence is offered to suggest infection by the olfactory route.

Thanks are due to the Boards of the Adelaide Children's Hospital Incorporated and the Royal Adelaide Hospital for permission to publish the cases, and to the honorary medical officers concerned for the use of the clinical records. We are especially grateful to Professor J. S. Robertson and Dr. K. Murray, of the Adelaide University Department of Pathology, for the use of the necropsy

material in one case. We thank Mrs. Helga Thiede for preparing the histological sections, and Mr. Ray Boyd for assisting with the illustrations.

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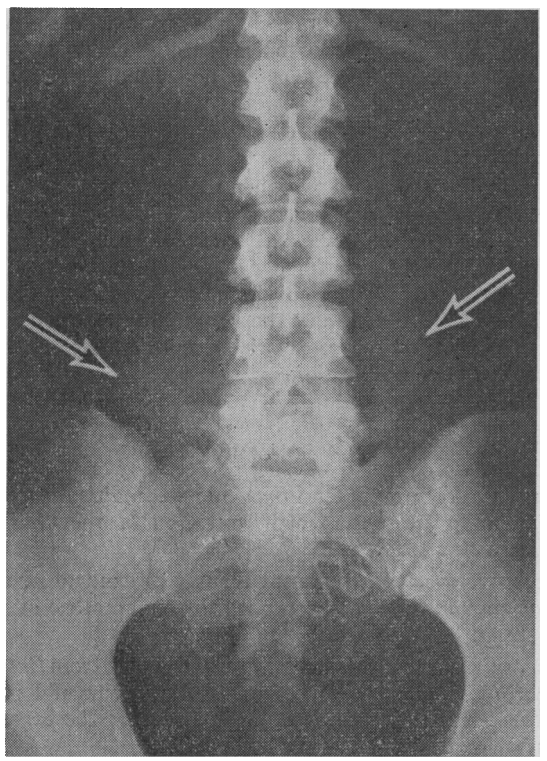
Medical Memoranda

Retention of Intrauterine Contraceptive Device during and after Delivery

Brit. med. J., 1965, **2**, 742

The use of intrauterine contraceptives is becoming more widespread. It is not yet known how they function but if one should fail there is ample evidence to show that it is unlikely to have an adverse effect on the ensuing pregnancy. (*Lancet*, 1964).

Armstrong and Andreson (1959) reported the first case of a device retained *in utero* until the third stage of labour—a Gräfenberg ring which was passed embedded in the placenta. Before this Zakin *et al.* (1955) had described cases where the device was spontaneously extruded or had been removed during pregnancy without harming the foetus.



Radiograph showing pregnancy (arrowed) superimposed on loop.

Oppenheimer (1959) confirmed the absence of ill-effects on the foetus, as did Hall and Stone (1962), who included an ectopic pregnancy in their series. Kwee (1964) reported two cases where a plastic loop was expelled at delivery: the one where the first of twins was a fresh stillbirth and the other encountered in a molar pregnancy. He does not attribute either of these events to the loop.

There is as yet, it seems, no report in a British journal of a device being retained *in utero* after delivery and requiring removal several months later.

CASE REPORT

A married woman, aged 26 and with four children, disliked the side-effects of oral contraception and was fitted with a Lippes loop in December 1962. Initially this was very satisfactory; she was quite unaware of its presence and her menstrual and marital life were normal.

In May 1963 an acute attack of lower abdominal pain was diagnosed as a pelvic infection and treated with antibiotics, though the patient suspected that she was pregnant. Three months later a radiograph showed a pregnancy superimposed on the loop (see Fig.) and in January 1964 she had a spontaneous vertex delivery of a female infant weighing 7 lb. 5½ oz. (3.3 kg.). The baby appeared normal in all respects and has since thrived.

The loop could not be found in the placenta or membranes, and a radiograph taken four days after delivery showed it to be still *in utero*. In June 1964 she was seen at the gynaecological clinic. Pelvic examination revealed no abnormality apart from slight tenderness on mobilizing the uterus. There was no tubal filling on hysterogram but the loop was easily seen. It was finally removed under anaesthesia in July 1964, six months after delivery. Endometrial curettings taken at the same time showed normal proliferative endometrium. The following day there was no pain on pelvic examination and the patient was sent home. When seen at the follow-up clinic she was using oral contraceptives and appeared well.

COMMENT

This case confirms previous reports that retention of an intrauterine contraceptive device is unlikely adversely to affect a pregnancy. It also demonstrates that should it not be passed at delivery it may be left *in utero* for a considerable time without causing any local or general reaction.

I am indebted to Mr. R. C. Cummin for permission to publish this case.

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